THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 25, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR: DR. DAVID J. HILL
SIGNATURE:
DEPARTMENT: MEDICINE
ADDRESS: F4-104 SJHC 268 GROSVENOR ST
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Location of experimental work to be carried out: Building(s) LHRI - SJHC Room(s) F4-124

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): see attached pages.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Brenda Strutt
Astrid Chamson-Reig
Aaron Cox
Christine Beamish
Michelle Durst
Kelly Weese

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? O YES Ø NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES O NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? Ø YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes Ø No		Not applicable
Rodent	Ø Yes O No	mouse/rat pancreas	2005-013-05
Non-human primate	O Yes Ø No		
Other (specify)	O Yes Ø No		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes Ø No		
Rodent	O Yes Ø No		
Non-human primate	O Yes Ø No		
Other (specify)	O Yes Ø No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate HC or CFIA containment level required Ø1 O2 O3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? Ø YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O1 O2 O3
Human Blood (fraction) or other Body Fluid	plasma from children - clinical trial	O Yes Ø No		O1 Ø2 O3
Human Organs or Tissues (unpreserved)		O Yes O No		O1 O2 O3
Human Organs or Tissues (preserved)		O Yes O No		O1 O2 O3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES Ø NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below O NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ✓NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ✓YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used WISTAR RAT, C57Bl/6 mice, BalbC mice NOD, NOD-SCIDmice.

6.3 AUS protocol # see attached

6.4 Will any of the agents listed be used in live animals ✓YES, specify: BrdU STZ Tamoxifen O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES ☑NO
- Pound source cats O YES ☑NO
- Cattle, sheep or goats O YES ☑NO
- Non- Human Primates O YES, please specify species _____ ☑NO
- Wild caught animals O YES, please specify species & colony # _____ ☑NO
- Birds O YES ☑NO
- Others (wild or domestic) O YES, please specify _____ ☑NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? ☑YES O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) cholera toxin
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin 250µg/kg

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ☑NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ☑ NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will the agent be imported? O YES, please give country of origin_____ ☑ NO
If no, please proceed to Section 10.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal pathogens? O YES O NO

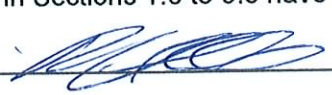
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE 

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ☑ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, permit # if on-campus Dec 16, 2008 JV
O NO
O NOT REQUIRED

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: 21/11/08

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: Dec 16, 2008.

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Title of Grants:

1-Pancreatic Beta cell renewal in development: Implications in diabetes
Protocol # 2002-114-12
Summary of the project: As the the numbers of diabetic patients are increasing worldwide the necessity of finding alternative treatments to the ones we have nowadays is crucial. Therefore, understanting the mechanisms by which beta cells start to lose the battle to maintain a normal secretion of insulin will allows us develop strategies to improve the maintaince, the generation or re-generation of healthy producing insulin cells.
One way to pursue this is to learn step by step how beta cells respond after a mild chemical insult by partial or total recovery. Our aim is to characterize in vivo the factors that are involved in the process. Strategies used in other organs to stimulate neogenesis are going to be tested in this model . We also want to optimize cell culture methods, in vitro, in order to use it as an alternative source to expand and/or obtain funcional islets for transplantation.
W e will study the effects of a restricted protein diet during pregnancy and lactation and its effects on regeneration on the pancreas of the pups when exposed to stz after birth (rats and mice).
This study will help us understand the mechanism by which beta cells regenerate in vivo .

STZ: rats (70mg/kg) i.p. (once)
STZ: mice (35 mg/kg) i.p. (5 consecutive days)

2-Growth Factors and Beta cell neoplasia
Protocol # 2005-013-05
Summary of the project: Our main objective is to determine the presence of primitive undeveloped cells within the pancreas (organ that controls glucose metabolism). We will study a genetically modified mice by increasing specific genes that are important during development of insulin-producing cells in the pancreas. We will also use a rat model to study the regeneration of the pancreas after damaging insulin producing cells at day 4 after birth,. We intend to learn about the internal cellular mechanisms that will control the growth and maturation of these cells. The benefits of this study will help us understand how cells in the pancreas grow and mature. We will utilize this knowledge to improve the current techniques of transplantation. Our main objective is to determine the presence of primitive undeveloped cells within the pancreas (organ that controls glucose metabolism). We will study a genetically modified mice by increasing specific genes that are important during development of insulin-producing cells in the pancreas. We will also use a rat model to study the regeneration of the pancreas after damaging insulin producing cells at day 4 after birth,. We intend to learn about the internal cellular mechanisms that will control the growth and maturation of these cells. The benefits of this study will help us understand how cells in the pancreas grow and mature. We will utilize this knowledge to improve the current techniques of transplantation.
Mice will be used as an accepted model for transgenic manipulation. Rats are going to be used to study the ontogeny of pancreatic regeneration after an insult (STZ) in a 4 day old neonate rat. After STZ injection rats were followed until 10 days after weaning. Animals will be injected i.p. with (5- Bromo—2 deoxyuridine) (BrdU, 50 mg/kg B.W.) 2-4 hours before euthanasia. BrdU is a thimidine analogue that labels replicating cells within S phase of the cell cycle.
STZ: rats and mice (70mg/kg) i.p. (once)
BrdU: rats and mice (50 mg/kg) (once)
3-Fetal programming and pancreatic development

Protocol#: 2006-088-10
Summary of the project: Programming of both quantity and quality of pancreatic B cells occurs in the third trimester in human and can be modeled in the neonatal mouse and rat.
Normal □ cell ontogeny involves a turnover of cells as a result of a balance of cell replication, islet neogenesis and programmed cell death, and this is independent on appropiate nutrition in the fetus an the neonate. Disbalance of these parameters may yield an adult population of □ cells that are inappropriately responsive to metabolic challenge.
Previously in collaboration with Dr. B. Reussens of the University of Leuven, Brusselles, we have shown that a low protein diet may induce long-term changes in the proliferative cell cycle kinetics and rates of developmental apoptosis in the beta cell cycle, either during fetal life, neonatal development, or both. We also have preliminary evidence that sulphated amino-acids such as taurine may reverse changes in beta cell proliferation and apoptosis caused by nutritional deprivation in utero, creating strategies for intervention in IUGR to reduce the risk of later type II diabetes
Firstly, we hypothesize that changes in the reduction of proteins during pregnancy may be responsible of altering the normal programming of the pancreas with beta cells that are not appropiate to respond to the metabolic challenges of adulthood.
Secondly, we hypothesize that many of the adverse effects of the low protein diet (LP) on □ cell biology may be reversed by the addition of supplements such as the amino acid Taurine or other sulphur amino-acids.
Thirdly, treatment with STZ in early in mice treated with a low protein diet will not allow pancreatic regeneration
Three hour before sacrifice either the pregnant dams or the postnatal litter members will be injected i.p. with *5-bromo-2-deoxyuridine (BrdU, 50 mg/kg body wt)*. The diets are isocalorific with equivalent fat contents, the balance of calories being supplied as carbohydrate in the low protein.
Same dietary approach e will be conducted on Balbc mice. Mice at birth were also inyected with *35mg of STZ , i.p. at birth for 5 consecutive days* . Pancreata will be collected at sacrifice at PN day 14 -21 and 44.
STZ: mice (35 mg/kg) i.p. (5 consecutive days)
BrdU: rats and mice (50 mg/kg) (once)

4- Control of regeneration in the endocrine pancreas (part I):

Protocol #: 2006-066-11
Summary of the project: The pancreas is one of the organs that is involved in the regulation of glucose. The cells that produce the factor (insulin) that maintains normal glucose levels in circulation are called beta cells and are the major compartment of the islets (cluster of cells within the pancreas)
The failure in the production of insulin by the damage of these cells leads to type I diabetes.
Previously we have shown that young rodent have the capacity to regenerate these cells after an injury (chemical insult).
The purpose of this study is to understand the underlying mechanisms by which beta cell regenerate in early life and to identify the elements which become rate limiting with age.
We also know that there is an increased growth of capillaries in the region of injury that helps repair the area. Therefore, we are interested in the involvement of this population of cells called endothelial progenitor cells that reside in the pancreas and can be mobilized after a chemical insult such as Streptozotocin (Stz) and its importance during regeneration.
We will investigate whether a local increase of certain protein called glucagon-like peptide 1 (GLP-1) may be a major driver in this regenerative process.

As our final objective we shall use genetic manipulation of pancreatic cell types to identify which cell population has the lead role in regeneration. To activate the transgene in the vav-cre mice, tamoxifen will be used 0.96mg/day (20mg/ml) diluted in 49ul IP for 5 consecutive days. Adult 90 days old vav-cre mice will be treated with a single dose of Stz (70mg/kg) and followed every two days for 1 week and then at 14 and 21 days (6 animals per time point). The results will be compared with neonatal mice. These experiments will determine if the changes in endothelial progenitor cells during regeneration are age related.
NOD scid mice (stz or not stz treated) will receive bone marrow-derived stem cells from either neonatal or adult vav-cre mice, in order to facilitate regeneration.

STZ: mice (70mg/kg) i.p. once
BrdU: mice (50mg/kg) (once)
Tamoxifen: mice (0.96mg/day (20mg/ml) diluted in 49ul IP for 5 consecutive days)

5- Growth Factors and Beta Cell Neoplasia

Summary of the Project: Our main objective is to determine the presence of primitive undeveloped cells within the pancreas, and study the ability of these cells, *in vitro*, to give rise to new functional islets. Islets isolated from the pancreata of neonatal or adult mice are maintained in primary cultures in a defined cell culture medium, the components of which are insulin, dexamethasone, epidermal growth factor and cholera toxin (100ng/ml). This medium promotes the dedifferentiation of the endocrine cells into epithelial cell monolayers to enrich for progenitor cells. After 4 weeks of culture, the cells are harvested and replated onto a laminin-rich extracellular matrix in medium containing FGF-7 and IGF-II. Over the course of the next several weeks, new neo-islets are formed. The lab is involved in trying to characterize the function of these neo-islets, and look at the factors which can cause them to mature into functional islets. The benefits of this study will increase our understanding of how islets develop, and may be useful in developing new strategies for islet transplantation in the diabetic patient.

References:

1. Transdifferentiation of human islets to pancreatic ductal cells in collagen matrix culture. Yuan, S et al. Differentiation 61:67-75. 1996
2. Factors mediating the transdifferentiation of islets of Langerhans to duct epithelial-like structures. Wang, R et al. Journal of Endocrinology 171:309-318. 2001
3. Stimulation of cAMP signaling allows isolation of clonal pancreatic precursor cells from adult mouse pancreas. Yamamoto, T et al. Diabetologia 49:2359-2367. 2006

Cholera toxin: 100ng/ml in tissue culture medium
0.5mg ordered at a time, this is probably enough to last 1 year.